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FORMULATION AND EVALUATION OF LIQUID CRYSTALLINE NANOPARTICLES FOR THE TREATMENT OF DIABETIC RETINOPATHY

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ABSTRACT

The rationale of the research was to formulate and evaluate a novel triamcinolone loaded liquid crystalline nanoparticles delivered through periocular sub conjunctival route for the treatment of diabetic retinopathy. The liquid crystalline nanoparticles were prepared by fragmentation of bulk cubic gel by high pressure homogenization method. The drug was loaded to the optimized formulation and characterization of triamcinolone loaded liquid crystalline nanoparticles were studied. FTIR was carried out to identify their functional groups and DSC was carried out to determine their melting point. The particle size, poly dispersity index and zeta potential obtained for the optimized formulation was found to be 6.626 (d.nm), 0.241 and - 29. 31mV and for the formulation loaded with triamcinolone was found to be 5.352 (d.nm) 0.243 and – 31.3 mV respectively. The pH of the optimized formulation was found to be 6.87 and for drug loaded formulation was found to be 7.36. The encapsulation efficiency of triamcinolone loaded liquid crystalline nanoparticles was 84.3% indicating most of the drug was encapsulated. The scanning electron microscopy and X-Ray diffraction studies was done to know the surface morphology of the nanoparticles and crystallinity of the drug, polymer and the liquid crystalline nanoparticles. The drug release from the liquid crystalline nanoparticles shows greater release till 20 h when compared with pure triamcinolone solution. The stability studies were done for the optimized formulation and the formulation loaded with triamcinolone at 25°C, 40°and 5°C. Hence, it was

concluded that the triamcinolone loaded liquid crystalline nanoparticles can be used for the treatment of diabetic retinopathy through periocular subconjunctival route.

KEYWORDS: Triamcinolone, Periocular Subconjunctival Route.

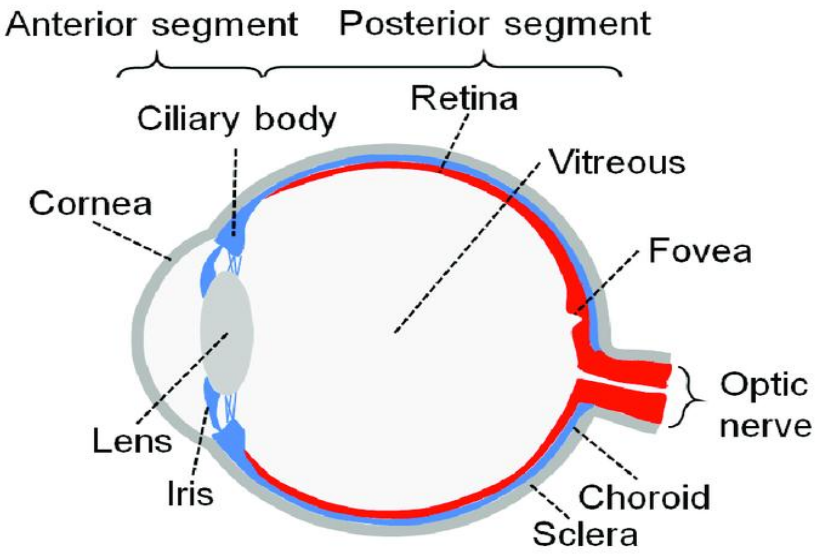
INTRODUCTION

Diabetes mellitus is a chronic disease that affects billions of 50 people all over the world. The disease can be classified into two chronic forms: Type I and Type II diabetes. Type I is because of the destruction of pancreatic islet beta cells with subsequent insulin deficiency. Type II, is mainly due to insulin resistance.

Diabetic retinopathy (DR) is one of the critical complications of diabetes mellitus (DM). Diabetic retinopathy (DR) has been recognized as the most significant damage that results in impaired vision and also causes vision loss.

Hyperglycaemia causes an increased activation and upregulation of VEGF, erythropoietin, growth factors, oxidative stress, protein kinase C, the polyol pathway, and advanced glycation end-products, among other factors and pathways.

Through these multiple mechanisms, hyperglycaemia induces apoptosis, inflammation, vascular changes, and angiogenesis. Currently, most therapeutic agents of eye diseases affecting the posterior segment of the eye are administered intravitreally. Topical application to the anterior eye has been proven successful in the treatment of diseases owing to easy access to the target site.



However, the adoption of mechanisms in ensuring topical drug penetration to the posterior eye presents numerous challenges. For the treatment of diabetic retinopathy several routes have been used for the drug administration like intravitreal, vitrectomy, laser photocoagulation, periocular route.

Among these periocular routes is the promising route for drug delivery system and it is having a large surface area for drug administration. The periocular route is divided in to subconjunctival, sub tenon, peribulbar route among this subconjunctival route is used for drug delivery.

The drugs which are already in market for the treatment of diabetic retinopathy like bevacizumab, ranibizumab, triamcinolone, dexamethasone, pegaptanib are formulated and given through intravitreal injection.

A wide range of Nano lipid carrier (NLC) can be used for topical application of drug.NLCs are the new generation of lipid nanoparticles, attracting major attention as novel colloidal drug carriers for topical use. In this project Nanostructured lipid carrier (NLC) were prepared to enhance and improve the ocular delivery of triamcinolone acetonide.

Triamcinolone acetonide is the most common corticosteroid used in the management of DR by intravitreal injection, since it is the most efficient approach to deliver the drug to the posterior segment of the eye. The common dose is 4.0 mg/0.1 mL, however higher doses of 20–25 mg/0.2 mL have also been reported.

Corticosteroids are a class of drugs with high anti-inflammatory activity. These drugs also provide the ability to reduce vascular permeability and reduce the blood–retinal barriers breakdown, to down-regulate VEGF expression and/or production.

Triamcinolone acetonide is being used for intravitreal injection to achieve a higher intraocular concentration in the post-segment of the eye. The NLC loaded with triamcinolone drug can penetrate through the cornea because of low molecular weight of drug and it is highly potent. The drug can accumulate in the target tissue, the pharmacological effect is shown in the posterior segment of the eye.

Through periocular route, delivery of drug level to the posterior segment of the eye is greater compared with topical or systemic mode of drug administration. In this research the periocular part of sub conjunctival route has been chosen for the drug administration.

MATERIAL AND METHODS

Materials

List of chemicals used

Sl. No	Chemical	Source
1	Poloxamer 407	SIGMA life science, USA
2	Monoolein	Tokyo chemical industry co., ltd., Japan
3	Triamcinolone	Tokyo chemical industry co., ltd., Japan
4	Milli pore water	Merck Ltd, Mumbai

List of equipment's used

Sl. No	Name of the Equipment	Models
1	Weighing balance	Shimadzu corporation, Japan
2	Homogenizer	PT 1600 EPolytron, USA
3	Magnetic stirrer	REMI 1 MLA, India
4	Water bath	Tempo instruments and equipment's Pvt. Ltd, India
5	Heating mantel	Skylab corporation, India
6	Differential scanning calorimetry	DSC 60, Shimadzu, Japan
7	FT-IR spectrometer	8400S Shimadzu, Japan
8	KBr Press	Model M-15 Techno search instruments, India
9	Probe Sonicator	Sonics vibra cell, USA
10	Particle size analyser	Malvern instruments, UK
11	pH meter	Systronics, India
12	Scanning electron microscopy (SEM)	JSM-IT300
13	X- Ray diffractometer	Bruker, AXS/8, Berlin, Germany
14	UV-Visible Spectrophotometer	1800, Shimadzu, Japan
15	UFLC, UV- Visible / PDA Detector	1800, Shimadzu, Japan
16	Stability	Thermolabs humidity chamber, Mumbai

Analytical method

Preparation of mobile phase

The mobile phase consisted of methanol and HPLC grade water (45: 55), filtered through 0.45 µm membrane filter and used for analysis.

Preparation of triamcinolone standard stock solution

The standard stock solution of triamcinolone was prepared by taking 10mg of pure drug in 10ml volumetric flask and dissolved with mobile phase consisting of methanol and HPLC grade water in the ratio of (45:55) and made up to the mark with mobile phase. Further serial dilutions were made to get 10, 20, 30, 40, 50 ng/ml.

Preparation of liquid crystalline nanoparticles

Liquid crystalline nanoparticles were prepared by fragmentation method. Glyceryl monooleate (monoolein) and poloxamer 407 in different weight ratios were first melted at 60^{o}C in a hot water bath. Milli-Q deionized water was added gradually to the solution and stir continuously using magnetic stirrer under 300rpm and vortex mixed for 1 min to achieve a homogenous state. After equilibration for 48 h at room temperature, an optically isotropic cubic- phase gel was formed. Subsequent fragmentation with milli-Q water to form a crude dispersion was performed by intermittent probe sonication at 25^{o}C for 5 min. The crude dispersion was finally homogenized by passing ten cycles through a high- pressure homogenizer at 689/ 25^{o}C to obtain an opalescent dispersion of the cubic nanoparticles.

Characterization of liquid crystalline nanoparticles

Fourier Transformed Infrared (FT-IR) Spectroscopic analysis

Fourier transform infrared analysis was conducted to verify the interaction between drug and polymer. The sample powder was dispersed in KBr powder and drug with ratio of 1:3 and were prepared by applying 600 kg/cm^{2} pressure. The spectral measurement was obtained by powder diffuse reflectance on a FT-IR spectrophotometer (Shimadzu, Model 8400, Japan), in a wave number region 4000 cm^{-1} to 400 cm^{-1}.

Differential scanning calorimetry (DSC)

Differential scanning calorimetry was performed for pure drug and its formulations. Calorimetric measurements were made with empty cell (high purity alpha alumina discs) as the reference and pan press was done. Spectral measurements were obtained on DSC- 60 at a temperature range of 20- 200°C. DSC analysis measures the amount of energy absorbed or released by a sample when it is heated or cooled, providing quantitative and qualitative data on endothermic and exothermic processes. The energy was measured as J/Kcal.

Particle size, zeta potential and polydispersity index measurement

The mean particle size, polydispersity index (PI) and zeta potential (ZP) were determined by photon correlation spectroscopy (PCS) with a zetasizer Nano ZS (Malvern instruments, UK) at 25^{o}C using disposal polystyrene cell and disposal plane folded capillary zeta cells. For particle size measurements of lipid nanoparticle dispersions refractive index was set as RI= 1.456. The laser diffraction size analysis was performed by a Mastersizer Hydro 2000MU (Malvern Instrument, UK).

pH

The pH of prepared formulations was determined using a calibrated pH meter. It is one of the most important parameter involved in the ophthalmic formulation. The two areas of critical importance are the effect of pH on solubility and stability. The pH of the ophthalmic formulation should be such that the formulation will be stable at that pH and at the same time there would be no irritation to the patient upon administration of the formulation. Ophthalmic formulations should have pH range in between 6.9 to 7.4.

Preparation of triamcinolone- loaded ophthalmic cubic nanoparticles

The optimized formulation of cubic nanoparticles prepared by fragmentation method was evaluated for ocular drug delivery using triamcinolone as a model drug. Required quantity of drug was added to the deionized water and melted at 60^{o}C for complete dissolution of drug. The aqueous solution of triamcinolone was then sterilized by autoclaving at 120^{o}C. This sterilized solution was then added to the crude dispersion (monoolein and poloxamer solution) under aseptic condition to for a phase gel followed by pre-mixing by high pressure homogenizer and probe sonication to get a triamcinolone- loaded ophthalmic cubic nanoparticles.

Characterization of triamcinolone loaded ophthalmic cubic nanoparticles

Drug encapsulation efficiency

The drug encapsulation efficiency (EE) were determined by ultrafiltration. One-milliliter triamcinolone cubosomes were added into the upper chamber of a centrifuge tube fitted with an ultrafilter (Pall Laboratories, 2.5 kDa, Germany), which was then centrifuged at 4000 rpm for 10 min. The amount of triamcinolone loaded in the cubosomes was calculated as the difference between the total amount used in formulation of the cubosomes and the amount in the filtrate, which was determined by ultraviolet-visible spectrophotometry at 239 nm. The drug encapsulation efficiency was calculated from the following equation

$$\text{Encapsulation efficiency} = \frac{\text{Total drug} - \text{Free drug}}{\text{Total drug}} \times 100$$

Scanning electron microscopy (SEM)

The surface morphology of nanoparticles was recorded by using SEM. Appropriate samples were mounted on an aluminum stub with double-sided adhesive tape. The tape was first firmly attached to the stub and the sample powder was scattered carefully over its surface. The stub with the specimen was then sputter coated with a thin layer of gold to make the specimens conductive. The processed specimen was subjected to SEM analysis.

X-Ray Diffraction

In X-ray diffraction studies, an automatic x-ray diffractometer equipped with a PW R30 x-ray generator was used. The dry sample powder was pressed into pellets and X-ray diffraction spectra were recorded using nickel-filtered Cu kα1 radiation having a wavelength of 1.5106 Å, operating at 35 kW and 20 mA. X-ray diffractograms were obtained at a scanning rate of 1 degree (2θ) per minute.

***In vitro* drug release**

Formulation equivalent to 100 mg of drug was used. Dissolution studies were conducted in a dissolution apparatus using modified dialysis method. 5 mL of cubosome dispersion was placed in a dialysis bag (cellophane membrane, molecular weight cut off 10,000–12,000, HiMedia, India), which was then sealed at both ends. The dialysis bag was dipped into the receptor compartment containing the dissolution medium, 900ml of phosphate buffer (pH 7.4), which was stirred continuously at 50 rpm and maintained at 37°C. At selected time intervals, aliquots were withdrawn from the release medium and replaced with the same amount of phosphate buffer. The samples were assayed spectrophotometrically at 239 nm and the cumulative percentage release of drugs versus time was plotted.

Stability studies

Stability is defined as the extent to which a product retains, within specified limits and throughout its period of storage and use, the same properties and characteristics that it possessed at the time of its manufacture. Stability testing is performed to ensure that the drug products retain their fitness for use until the end of their expiration dates. Stability studies as per ICH guidelines were carried out on optimized formulation at 25°C, 40°C and 5°C.

Statistical analysis

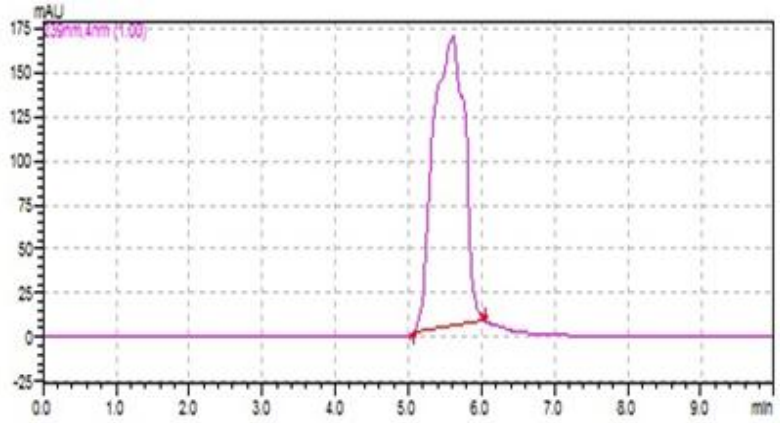
Raw data were analysed using the SPSS statistical software (version 11.0, SPSS, Inc.). Analysis of variance was done to determine the significance of the differences between groups; a P value <0.05 was considered statistically significant.

RESULT AND DISCUSSION

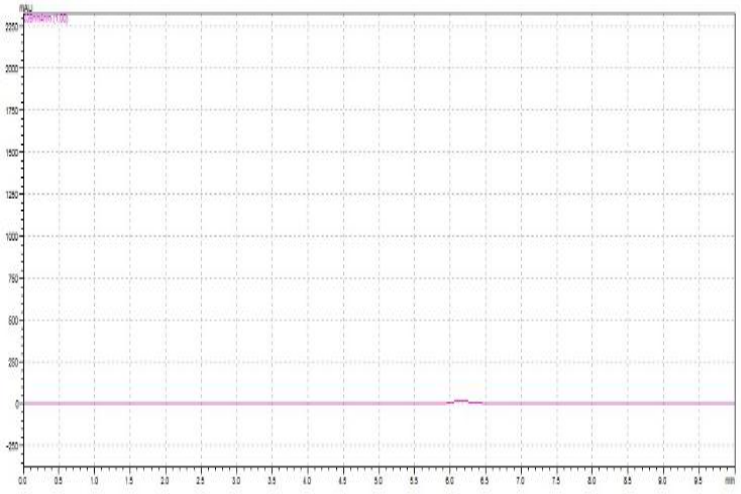
Method development by UFLC

In developing the method, systematic study of the effect of various parameters was carried out. Initially the solubility of the triamcinolone drug was determined. Then appropriate wavelength in UV region has been selected for the measurement of active ingredients in the proposed study. Initially, various mobile phase composition was tried to elute the drugs. Mobile phase and flow rate selection was based on peak parameters (height, capacity, theoretical plates, tailing or asymmetry factor, run time and resolution. In this method gradient elution with a mobile phase A (methanol) and mobile phase B (water) in the ratio of 45:55 v/v at the flow rate of 1ml/min and run time of 15 min were used. The optimum wavelength for detection was 239 nm at which better detector response for the drug was obtained. The retention time of triamcinolone was found at 5.6 min. Linearity was observed with a concentration range of 10-50 µg/mL with $R^2 = 0.99$.

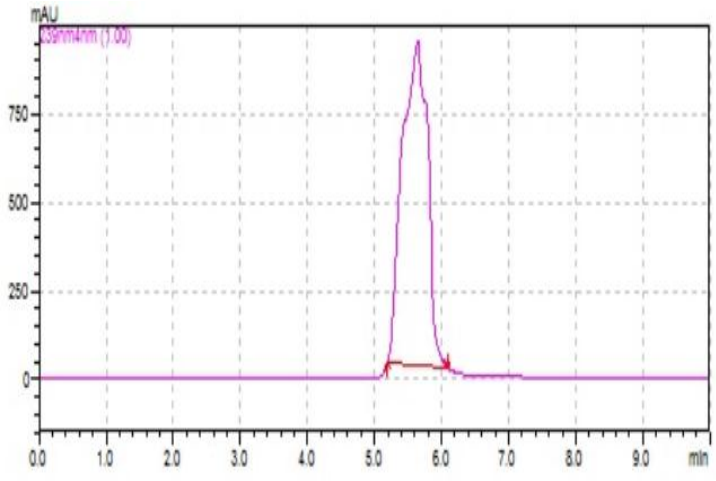
From the standard chromatograms various system suitable parameters were recorded.



Chromatogram of pure triamcinolone.



Chromatogram of liquid crystalline nanoparticles.



Chromatogram of liquid crystalline nanoparticles loaded with triamcinolone.

Peak results for standard triamcinolone.

Name of drug used	RT	Area	Peak start	Peak end	Tailing factor	Theoretical plates
triamcinolone	5.6	3978214	5.2	5.9	1.106	2400

Chromatographic conditions for triamcinolone.

Parameters	Conditions used
λ_{max}	239 nm
Column	Phenomenex C_{18} [250x 4.5 mm, 5 µm]
Mobile phase	Methanol: water (45:55)
Flow rate (mL/ min)	1ml/min
Injection volume	10 Ml
Regression equation y= mx+ c	Y=82969x – 88823
Slope, m	82969
Intercept, c	-88823

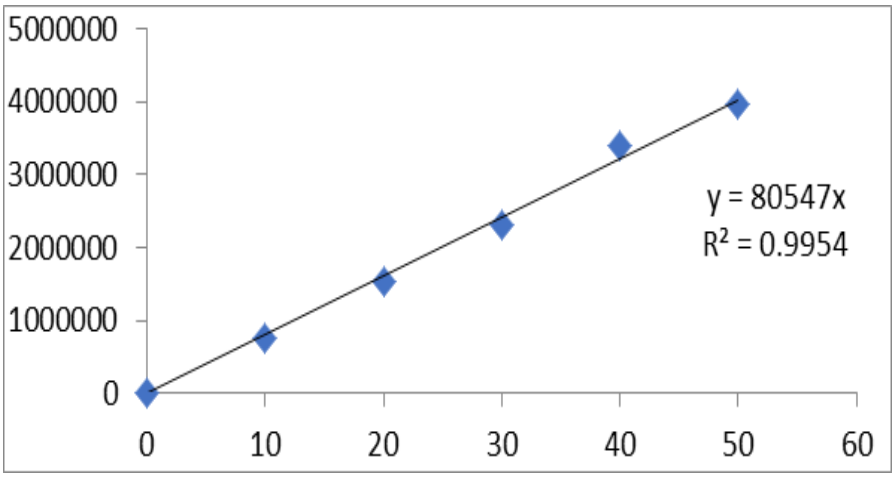
Linearity curve

The linearity of an analytical method is the ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range (5% to 50%) shown below.

Result for linearity

Level	Concentration in PPM	Area of triamcinolone
1	0	0
2	10	769872 ± 0.12
3	20	1526539 ± 0.15
4	30	2319872 ± 0.17
5	40	3406791 ± 0.021
6	50	3978214 ± 0.012

SD = Standard deviation n= 3



Calibration curve of triamcinolone

Preparation of liquid crystalline nanoparticles

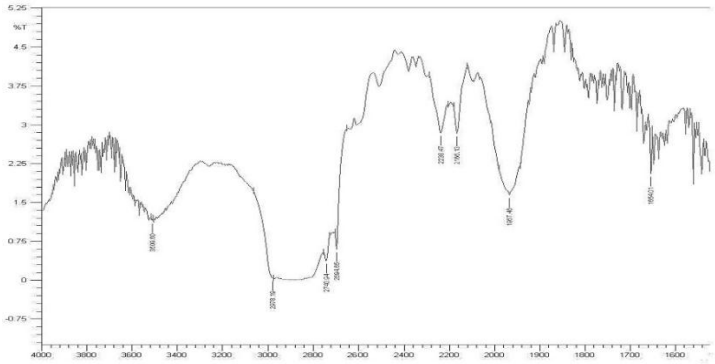
Several approaches have been employed to prepare cubic liquid crystalline nanoparticles. Fragmentation of cubic bulk gel by high energy dispersion like sonication and homogenization was used in this research. The preparation and characterization of cubic liquid crystalline nanoparticles was based on study with blank cubic phases and drug loading for optimized formulation. After equilibrate for 24hrs, clear and viscous gels formed from monoolein/ poloxamer 407. Cubic phase gel formed more rapidly when the ratio of poloxamer 407 increased up to 20%. The DOE method was used for the formulation, nine formulations of liquid crystalline nanoparticles were prepared using 3^2 full factorials with 2 factors and 3 levels.

Characterization of liquid crystalline nanoparticles

FT-IR studies

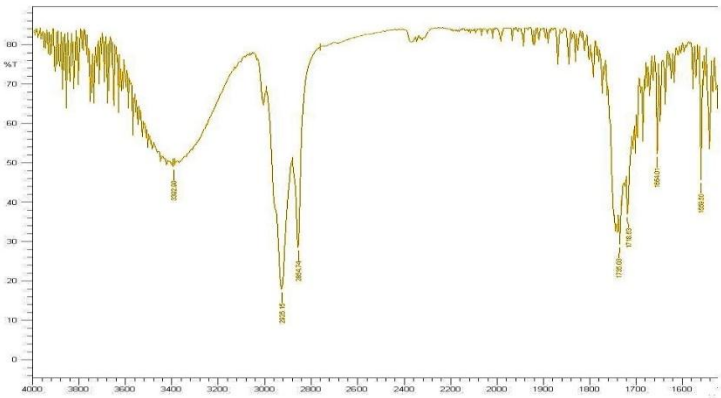
FT-IR analysis predicts the possible interaction between the drug, polymer and the formulation. The characteristic peak of triamcinolone, poloxamer 407 and monoolein were observed. There were characteristic peaks which are responsible for complex formation. The overlay spectra of triamcinolone, poloxamer 407, monoolein and formulation are shown.

FTIR- spectra of poloxamer 407



FT-IR- spectral data of poloxamer 407

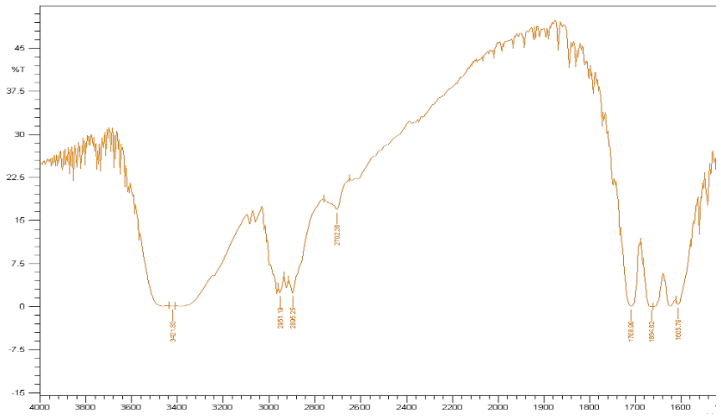
Sl. No.	Bond nature and attribute	Wavelength (cm^{-1})
1	C- H stretch aliphatic	2893.02
2	O- H bend	1355.86
3	C- O stretch	1124.42



FT-IR spectra of monoolein

FT-IR- spectral data of monoolein

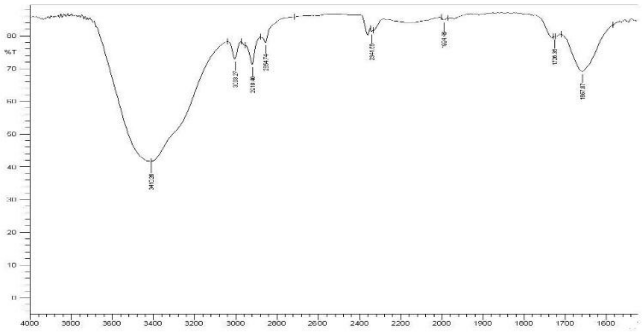
Sl. No.	Bond nature and attribute	Wavelength cm^{-1}
1.	–OH stretching	3,307
2.	–CH stretching	2,918
3.	–COO stretching	2,851



FT-IR spectra of triamcinolone.

FT-IR- spectral data of triamcinolone.

Sl. No.	Bond nature and attribute	Wavelength cm^{-1}
1.	O- H stretching	3387.65
2.	C= O stretching	1707.58
3.	C- H stretching	1614.34
4.	Carbonyl group of aliphatic ester bonds	1662



FT-IR spectra of liquid crystalline nanoparticle formulation.

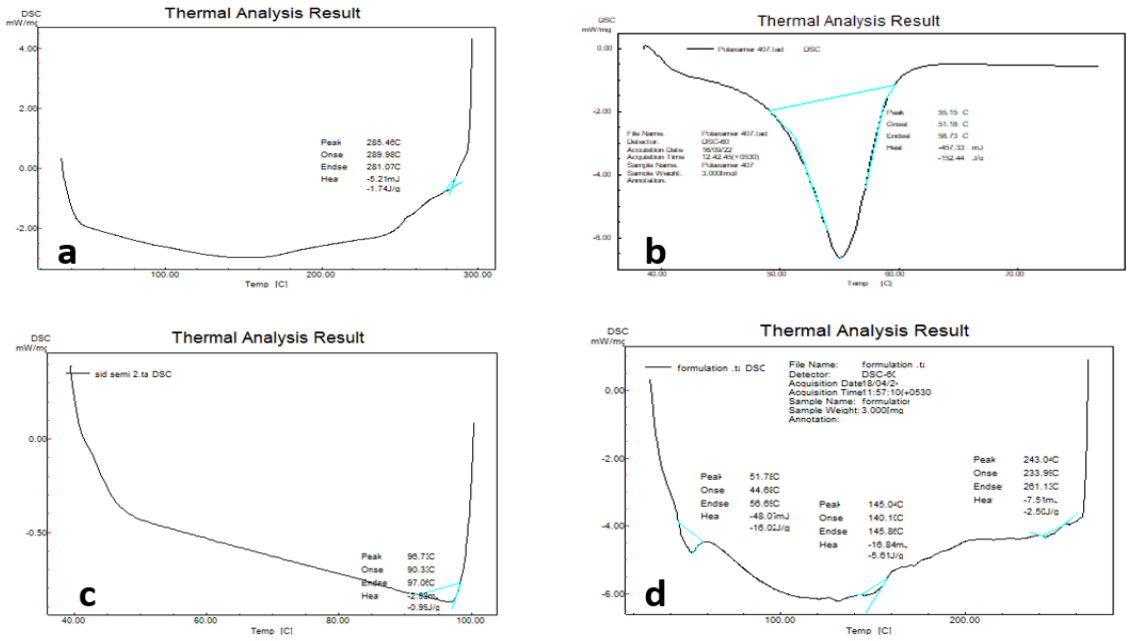
FT-IR- spectral data of liquid crystalline nanoparticle formulation.

Sl. No.	Functional group	Wavelength cm^{-1} of pure drug	Frequency of formulation
1	O- H stretching	3387.65	3562.57
2	C= O stretching	1707.58	1742.64
3	C- H stretching	2918	2554.74
4	Carbonyl group of aliphatic ester bonds	1662	1607.87

Differential scanning colorimetry studies

Thermogram of pure drug has shown a endothermic peak at 285°C, which corresponds to its melting point, polymer like poloxamer 407 shows a sharp endothermic peak at 55.15°C, which corresponds to its melting point and a lipid called monoolein shows a endometric peak

at 90°C, which corresponds to its melting point. The DSC thermograms revealed that there was significant difference between the drug and the excipients.



DSC thermogram of (a) Triamcinolone drug (b) Poloxamer 407(c) monoolein (d) liquid crystalline nanoparticles

Particle size, zeta potential and polydispersity index

The particle size is an important factor affecting the dispersion of ophthalmic formulations. Generally, the particle size of liquid crystalline nanoparticles that are able to penetrate through the cornea should be smaller than 200 nm with poly dispersity index. Zeta potential is used for the stability and bio- distribution of colloidal dispersion. High surface charges lead to electrical repulsion between particles and thus prevents aggregation. Zeta potential was found to be negative for triamcinolone loaded cubosome nanoparticles. Poloxamer 407 acts as stabilizer for cubic phase dispersions effectively and also preserve inner cubic crystalline structure of the particles. The responses obtained from the formulation using design of experiment is shown below.

Characterization of liquid crystalline nanoparticles.

Sl. No	Formulation	Particle size (d. nm)	Polydispersity index	Zeta potential mV
1	F1	7.016 ± 0.12	0.237 ± 0.21	- 36 ± 0.20
2	F2	9.574 ± 0.18	0.261 ± 0.13	- 31.3 ± 0.14
3	F3	38.23 ± 0.23	0.521 ± 0.15	- 40.6 ± 0.14
4	F4	33.65 ± 0.13	0.479 ± 0.17	- 35.6 ± 0.23

5	F5	7.127 ± 0.24	0.238 ± 0.23	- 37.4 ± 0.15
6	F6	4.674 ± 0.15	0.233 ± 0.18	- 30.6 ± 0.22
7	F7	8.384 ± 0.26	0.321 ± 0.19	- 29.3 ± 0.20
8	F8	15.59 ± 0.18	0.34 ± 0.25	- 40 ± 0.21
9	F9	8.544 ± 0.19	0.243 ± 0.21	- 36 ± 0.16

SD= Standard deviation n= 3

Anova responses

Anova for 2F1 model - Particle size.

Source	Sum of squares	df	Mean square	F- value	P- value	Remarks
Model	1051.99	3	350.66	9.61	0.0162	Significant
A- Monoolein	278.43	1	278.43	7.63	0.0397	
B- Poloxamer 407	552.52	1	552.52	15.14	0.0115	
A B	221.04	1	221.04	6.06	0.0572	
Residual	182.51	5	36.50			
Cor total	1234.51	8				

The Model F-value of 9.61 implies the model is significant. There is only a 1.62% chance that an F-value this large could occur due to noise.

ANOVA for response surface linear model – Polydispersity index.

Source	Sum of squares	df	Mean squares	F- value	P- value	Remarks
Model	0.0764	2	0.0382	11.32	0.0092	Significant
A	0.0158	1	0.0158	4.68	0.0737	
B	0.0606	1	0.0606	17.95	0.0055	
Residual	0.0203	6	0.0034			
Cor total	0.0967	8				

The Model F-value of 11.32 implies the model is significant. There is only a 0.92% chance that an F-value this large could occur due to noise.

Anova for 2F1 model – Zeta potential

Source	Sum of squares	df	Mean squares	F- value	P- value	Remarks
Model	108.44	3	36.15	8.52	0.0207	Significant
A	81.40	1	81.40	19.18	0.0072	
B	2.53	1	2.53	0.5973	0.4745	
A B	24.50	1	24.50	5.77	0.0614	
Residual	4.24	5	4.24			
Cor total	129.66	8				

The Model F-value of 8.52 implies the model is significant. There is only a 2.07% chance that an F-value this large could occur due to noise.

Results of Regression analysis of response – Particle size.

Response 1: Particle size	Value	F- value	P- value
R- Squared	0.85222	9.61	0.0162
Adj R- Squared	0.7635		
Pred R- Squared	0.6500		
Adeq R- Squared	8.4561		

The Predicted R^2 of 0.6500 is in reasonable agreement with the Adjusted R^2 of 0.7635; i.e. the difference is less than 0.2.

Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 8.456 indicates an adequate signal. This model can be used to navigate the design space.

Result of regression analysis of response – Polydispersity index.

Response 2: Polydispersity index	Value	F- value	P- value
R- Squared	0.7904	11.32	0.0092
Adj R- Squared	0.7206		
Pred R- Squared	0.4759		
Adeq R- Squared	9.0512		

The Predicted R^2 of 0.4759 is not as close to the Adjusted R^2 of 0.7206 as one might normally expect; i.e. the difference is more than 0.2. This may indicate a large block effect or a possible problem with your model and/or data. Things to consider are model reduction, response transformation, outliers, etc. All empirical models should be tested by doing confirmation runs.

Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 9.052 indicates an adequate signal. This model can be used to navigate the design space.

Result of regression analysis of response – Zeta potential.

Response 3: Zeta potential	Value	F- value	P- value
R- Squared	0.8363	8.52	0.0207
Adj R- Squared	0.7381		
Pred R- Squared	0.5729		
Adeq R- Squared	8.9678		

The Predicted R^2 of 0.5729 is in reasonable agreement with the Adjusted R^2 of 0.7381; i.e. the difference is less than 0.2.

Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 8.968 indicates an adequate signal. This model can be used to navigate the design space.

The application of factorial design yielded the following regression equations.

Particle size = 62.21102 + 58.86104 monoolein + 1076.20104 poloxamer – 929.21875 monoolein* poloxamer

Polydispersity index = + 0.298972 – 0.256667 monoolein + 2.51250 poloxamer

Zeta potential = - 25.55625 – 12.52083 monoolein – 262.18750 poloxamer + 309.37500 monoolein* poloxamer

Where negative values indicate a negative effect of a specific variable on the response factor and positive value indicates positive effect of a specific variable.

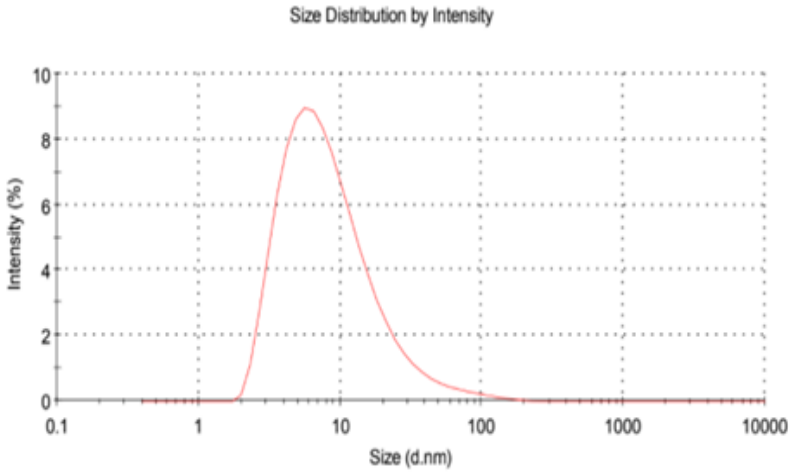
The regression equation depicts that the effect of monoolein and poloxamer 407 on particle size. It clearly indicates that at moderate concentration of monoolein and poloxamer 407 optimum particle size is obtained.

The polynomial regression results were expressed using Contour graphs, predicted & actual graphs and 3-D graphs.

Particle size

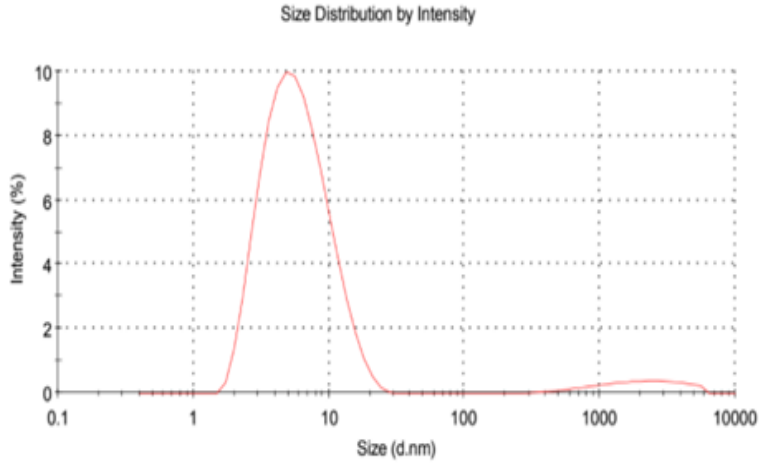
The particle size, polydispersity index and zeta potential of the optimized formulation was found to be 6.626 (d. nm), 0.241 and - 29. 31 and for the drug loaded formulation was found to be 5.352 (d. nm), 0.243 and – 31.3mV. This indicates, that the particles size of the triamcinolone loaded liquid crystalline nanoparticles can penetrate to the posterior segment of the eye through cornea and retinal barriers.

Blank formulation



Particle size of liquid crystalline nanoparticles.

Drug loaded liquid crystalline nanoparticles



Particle size triamcinolone loaded liquid crystalline nanoparticles.

pH

The pH of the optimized formulation was found to be 6.87 and the pH drug loaded formulation was found to be 7.1. Since, lacrimal fluid is alkaline, with a pH of 7.36, the variation of the pH often determines the speed and the quality of absorption of the drug and

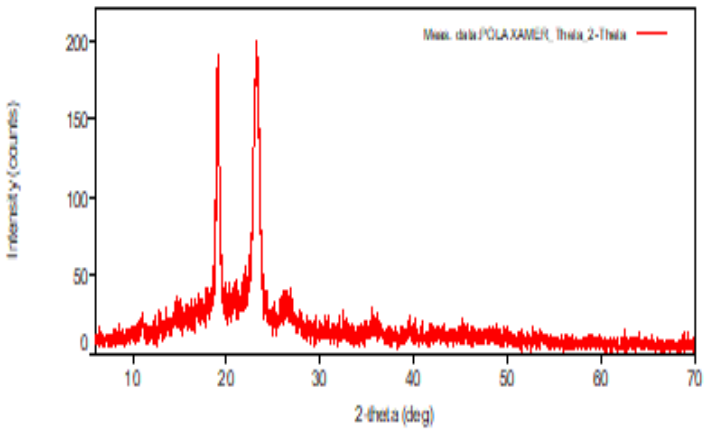
the amount of irritation which the patient experiences on instillation. Hence pH of the formulation was satisfactory.

Drug encapsulation efficiency

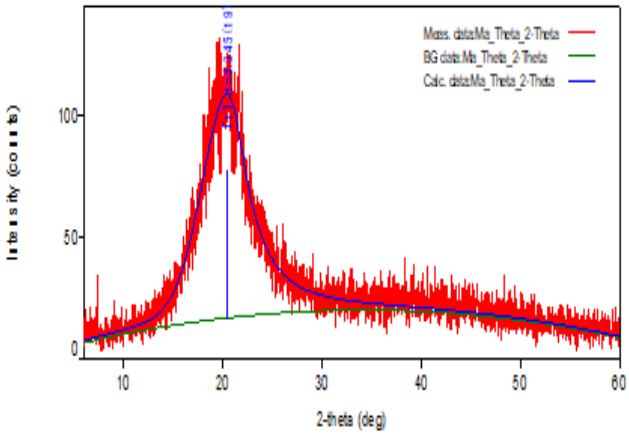
The encapsulation efficiency of the cubosomes was found to be 84.3%. This indicates that most of the drug was encapsulated in the liquid crystalline nanoparticles and it is satisfactory. The relative low encapsulation efficiency for hydrophilic drugs was attributed to the limited encapsulation capacity of the water channel in the cubosomes. The entrapment efficiency is satisfactory because of the lipid present in the formulation. Therefore, the higher encapsulation efficiency for triamcinolone may be ascribed to relatively sufficient water channel space for encapsulating drugs with a high potency of triamcinolone.

X-Ray Diffraction

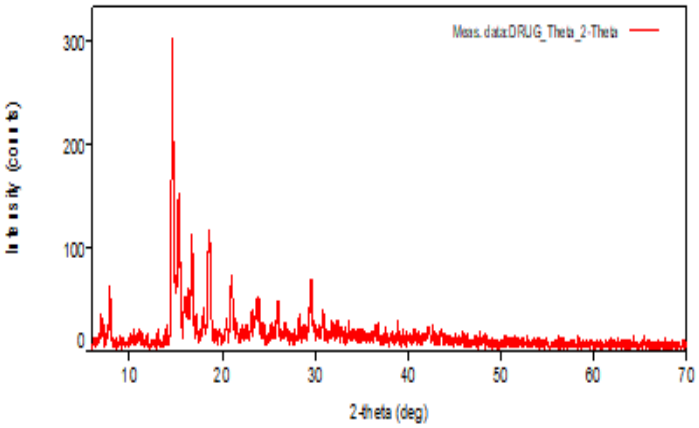
XRD analysis was done to evaluate the crystallinity of the formulation. The XRD spectra of pure triamcinolone drug, poloxamer 407, monoolein and the formulation are shown. The triamcinolone exhibits crystalline nature which was clearly represents. The characteristics peaks of poloxamer 407 shows more crystalline. The analysis liquid crystalline nanoparticles showed that the drug has been reduced into amorphous form and this is represented by reduction in the peak intensities. This showed that triamcinolone is reduced from crystalline form to amorphous form.



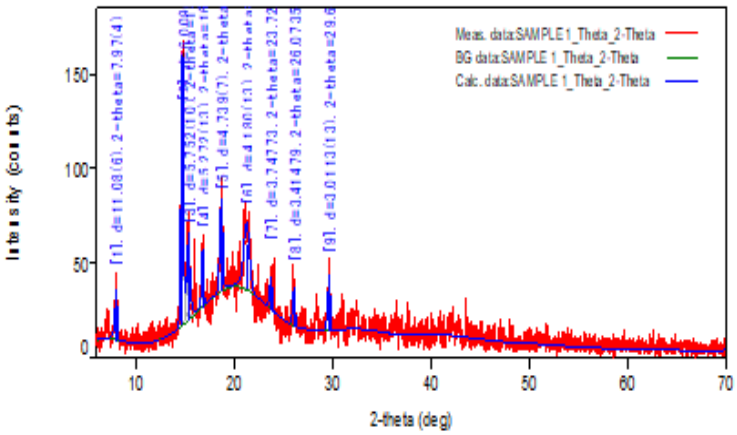
X- Ray diffractogram for poloxamer 407.



X- Ray diffractogram for monoolein.



X- Ray diffractogram for triamcinolone.

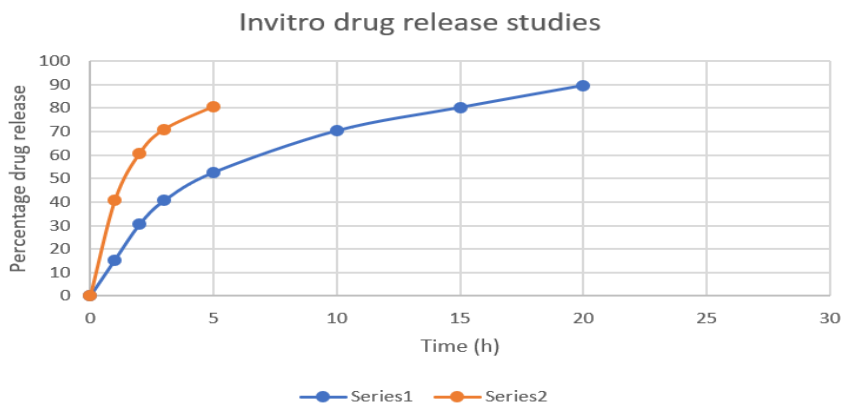


X- Ray diffractogram for liquid crystalline nanoparticles.

In vitro drug release studies

In vitro drug release profiles are reported for triamcinolone loaded cubosomes compared to triamcinolone solution. A hasty and complete release was observed from triamcinolone solution after 2 h. The release profile of triamcinolone loaded cubosomes was found to be

biphasic, with initial release is around 43.56 ±1.48% of drug during the first 1.5 h, followed by a moderately slow release of the remaining drug for a period of 20 h. The higher initial release is mainly due to the adsorbed drug or weakly bound drug to the relatively larger surface of cubosomes. The release of hydrophilic and hydrophobic drugs from cubosomes was previously reported as shown in below table.



Series 1: Triamcinolone solution Series 2: Triamcinolone loaded with cubosomes

***In vitro* drug release graph for liquid crystalline nanoparticles and pure drug**

***Invitro* drug release studies for pure drug and liquid crystalline nanoparticles formulation**

Time (h)	% Cumulative drug release ((Mean ± SD*)	
	Triamcinolone solution	Triamcinolone loaded cubosomes
0	0	0
1	40.8 ± 0.025	15.4 ± 0.032
2	60.7 ± 0.027	30.5 ± 0.05
3	70.9 ± 0.030	40.6 ± 0.047
5	80.6 ± 0.014	52.6 ± 0.034
10	-	70.4 ± 0.026
15	-	80.3 ± 0.015
20	-	89.4 ± 0.020

SD= Standard deviation n= 3

Stability studies

The stability studies were done for 1 months at 25°C, 40°C and 5°C. The stability of some formulations was changed and get separated due to temperature and the optimized formulation that contain triamcinolone has stable for 2 months and there is no separation seen in the formulation and no change in pH.

Stability studies at 25°C

Days	Formulation	pH	Separation
0 days	F1	6.62	No separation
	F2	6.94	No separation
	F3	6.83	No separation
	F4	6.92	No separation
	F5	7.02	No separation
	F6	7.23	No separation
	F7	7.31	No separation
	F8	6.92	No separation
	F9	7.12	No separation
15 days	F1	6.64	Separation
	F2	6.89	No separation
	F3	6.93	Separation
	F4	6.87	Separation
	F5	7.12	No separation
	F6	7.01	No separation
	F7	7.34	No separation
	F8	6.69	No separation
	F9	7.21	Separation
30 days	F1	6.90	Separation
	F2	6.87	No separation
	F3	6.97	Separation
	F4	6.85	Separation
	F5	7.31	No separation
	F6	7.24	Separation
	F7	7.34	No separation
	F8	7.21	No separation
	F9	6.93	Separation

Stability studies at 40°C.

Days	Formulation	pH	Separation
0 days	F1	6.64	No separation
	F2	6.93	No separation
	F3	7.23	No separation
	F4	7.32	No separation
	F5	6.87	No separation
	F6	7.32	No separation
	F7	6.86	No separation
	F8	7.23	No separation
	F9	7.32	No separation
15 days	F1	6.86	Separation
	F2	6.92	No separation
	F3	6.94	No Separation
	F4	6.89	Separation
	F5	7.32	No separation
	F6	7.21	No separation

	F7	7.36	No separation
	F8	6.68	No separation
	F9	7.24	Separation
30 days	F1	6.93	Separation
	F2	6.86	No separation
	F3	6.99	Separation
	F4	6.89	Separation
	F5	7.31	No separation
	F6	7.42	No Separation
	F7	7.45	No separation
	F8	7.31	No separation
	F9	6.93	Separation

Stability studies at 5°C.

Days	Formulation	pH	Separation
0 days	F1	7.02	No separation
	F2	7.23	No separation
	F3	7.31	No separation
	F4	6.92	No separation
	F5	7.12	No separation
	F6	6.62	No separation
	F7	6.94	No separation
	F8	6.83	No separation
	F9	6.92	No separation
15 days	F1	7.31	Separation
	F2	7.24	No separation
	F3	7.34	No separation
	F4	7.21	Separation
	F5	6.93	No separation
	F6	7.23	No separation
	F7	7.32	Separation
	F8	6.87	Separation
	F9	7.32	No separation
30 days	F1	6.86	Separation
	F2	7.23	No separation
	F3	7.32	No separation
	F4	6.87	No separation
	F5	7.32	Separation
	F6	6.86	No separation
	F7	7.01	No separation
	F8	7.34	No separation
	F9	6.69	Separation

The triamcinolone drug was added to the optimized formulation and kept for stability studies at 25°C, 40°C and 5°C. There is no separation occurs and no change in pH for 2 months.

Stability studies for optimized formula

Days	Formulations	pH	Separation
0	Triamcinolone loaded cubosomes	6.9- 7.4	No separation
15		6.91- 7.3	No separation
30		6.89- 7.2	No separation
45		6.87- 7.1	No separation
60		6.88- 7.4	No separation

SUMMARY

- The objective of the present work was to formulate and evaluate the liquid crystalline nanoparticles using triamcinolone as a drug. These were prepared and incorporated as eye drops.
- In HPLC the separations were achieved with Phenomenex C_{18} [250x 4.5 mm, 5 µm] column. The mobile phase methanol and HPLC grade water in the ratio of 45:55 v/v, with a flow rate of 1 mL/min and the eluent was monitored at 239nm. The selected chromatographic conditions were found to give effective separation of triamcinolone with retention time of 5.6 min. The linearity for triamcinolone was 10-50 µg/mL.
- Liquid crystalline nanoparticles formulations were prepared using different ratios of monoolein and poloxamer 407 and they showed good yield.
- The full 2^3 factorial design for the optimization of liquid crystalline nanoparticles was done using DoE software. Nine formulations were prepared among these the optimized formulation was obtained.
- The particle size, polydispersity index and zeta potential are taken as 3 responses for the optimization of formulation. The particle size of the optimized formulation was found to be 6.626, poly dispersity index to be 0.241 and zeta potential to be -29.31mV. The particle size of the liquid crystalline nanoparticles loaded with triamcinolone was found to be 5.352, poly dispersity index to be 0.243 and zeta potential to be -31.3mv. This might be mainly because of more anions present on the surface of the particles. The liquid crystalline nanoparticles were moderately stable.
- FT-IR spectra of prepared formulation shows that there is a significant change in the finger print region ie., 800- 3500 cm^{-1}. This confirms that the formation of bond between polymer and the drug.
- The SEM photography shows that optimized cubosomes were spherical and rod shape and the size of the nanoparticle was below 200nm.
- DSCclearly shows that there is a shift in the glass transition temperature to a lower temperature with decrease in the heat of fusion. This confirms that the crystalline nature

of the triamcinolone is reduced due to encapsulation of the drug in the liquid crystalline nanoparticles.

- XRD analysis clearly represents that there is a reduction in crystallinity of triamcinolone after it is homogenized and formulated.
- For the optimized formulation (F2) the drug encapsulation efficiency was done, in which the greater amount of drug was encapsulated in liquid crystalline nanoparticles.
- The optimized formulation of liquid crystalline nanoparticles loaded with triamcinolone (F2) and pure triamcinolone solution were subjected to invitro release studies. The triamcinolone loaded liquid crystalline nanoparticles shows maximum drug release for 20 hours.
- pH of the liquid crystalline nanoparticles was near to the lacrimal fluid pH of 6.9- 7.4 and this indicates there is no ocular irritation caused due to formulation.

CONCLUSION

- Liquid crystalline nanoparticles that contains triamcinolone with 4:1 ratio of monoolein and poloxamer 407 has shown good invitro release.
- Sustained release of triamcinolone drug till 20 h was achieved by delivering through liquid crystalline nanoparticle formulation.
- The particle size of liquid crystalline nanoparticles was in the range of below 200 nm hence it can be penetrating through the cornea to the posterior segment of the eye ie. Retina from periocular subconjunctival route.
- Hence it can be concluded that triamcinolone loaded liquid crystalline nanoparticles is suitable for the treatment of diabetic retinopathy.

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